Fibronectins: Multifunctional Modular Glycoproteins

RICHARD O. HYNES and KENNETH M. YAMADA
Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; and the Membrane Biochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Fibronectins are large glycoproteins that have been implicated in a wide variety of cellular properties, particularly those involving the interactions of cells with extracellular materials. These properties include cell adhesion, morphology, cytoskeletal organization, migration, differentiation, oncogenic transformation, phagocytosis, and hemostasis. During the past several years, investigations in many laboratories have analyzed the expression, functions, and structure of fibronectins. These studies have revealed that fibronectins have a complex molecular structure consisting of multiple specific binding sites, and the complex biological phenomena in which fibronectins participate can now be considered in terms of this structure.

In this brief article we will review the current understanding of the structure and properties of fibronectins. Because a number of comprehensive reviews on various aspects of fibronectins have been published (1–13), we shall focus on overall concepts, recent developments and promising future research directions in this rapidly expanding field.

Sources of Fibronectins

In vivo, fibronectins are found in body fluids (300 μg/ml in plasma, lesser amounts in other fluids), soft connective tissue matrices, and most basement membranes. Fibronectins are synthesized by a wide variety of cells in vitro (Table I). Fibroblasts and endothelial cells are major producers, but many other cell types, including some epithelial cells, synthesize fibronectin at lower levels. There are at least two types of fibronectin, termed plasma and cellular fibronectins, although there may well be multiple forms of cellular fibronectin. Cellular and plasma fibronectins, although distinguishable, are very similar in structure and properties (see below). One major source of plasma fibronectin appears to be hepatocytes (14, 15)[1] although endothelial cells (16–18) and macrophages (19–22) could also contribute, given their close association with the bloodstream.

Properties of Fibronectins

The basic properties of fibronectins are listed in Table II. The molecule is asymmetric and consists of two similar or identical subunits of molecular weight 220,000 ± 20,000 daltons held together by disulfide-bonding near their carboxyl termini. Biophysical measurements indicate that, although the molecule as a whole is flexible, it contains compact globular domains (23, 24). Electron microscopic results confirm the idea that the molecule is extended and flexible (25–27), although the globular domains have not yet been observed by this technique.

One important characteristic of fibronectins is that they are capable of interacting specifically with a wide variety of other macromolecules. Table III lists the various interactions, some of which have been studied in more detail than others. The best-established interactions, for which evidence exists both for their occurrence in vivo and for their specificity in vitro, are the interactions with gelatin and collagens, fibrin, factor XIIIa transglutaminase, heparin, and proteoglycans. In addition, it is clear that fibronectins interact with many cells; however, the molecular basis for this interaction remains unclear and is one of the major unanswered questions concerning fibronectins. The binding sites on fibronectins involved in many of these interactions have been identified and isolated, as will be discussed in a later section.

Although plasma fibronectin exists as a soluble protein, the typical appearance of cellular fibronectin is as a fibrillar extracellular matrix. The fibrils containing fibronectin can also contain collagens (28, 29) and proteoglycans (30–32). It is possible that interactions between fibronectin and other matrix molecules are important in the formation of these fibrils. The detailed molecular interactions involved in the formation of extracellular matrices and basement membranes are another area of active research.

FUNCTIONS OF FIBRONECTINS

Table IV summarizes the cellular properties in which fibronectins have been implicated. The most thoroughly studied, and perhaps most basic, function of fibronectins is in the adhesion to solid substrates. Numerous studies have reported that fibronectins promote the adhesion and/or spreading of cells on a variety of materials including plastic, collagen, gelatin, and fibrin (33–36). Cells that synthesize their own fibronectin do

This work was presented in a symposium on Fibronectin and Other Cell Anchorage Proteins at the Twenty-first Annual Meeting of the American Society for Cell Biology (Anaheim, California, November 1981).

[1] Wherever reference 15 appears, it refers to a paper by Tamkun, J. W., and R. O. Hynes, submitted for publication.

TABLE I

*Fibronectin Synthesis In Vitro**

Cell type	Comments
Fibroblasts	Often decreased after oncogenic transformation
Endothelial cells	High rate of synthesis, large proportion secreted in vitro
Chondrocytes	Amount correlates inversely with differentiation
Myoblasts & myotubes	Quantity appears to differ according to source of cells; transformed cells have less; myotubes often have less than myoblasts
Macrophages	All or most is secreted
Hepatocytes	All or most is secreted plasma fibronectin
Amniotic cells	Amniotic FN is more heavily glycosylated
Glial cell lines	Glial cells in vivo probably do not synthesize FN.
Intestinal epithelial cells	Small amounts only
Mammary epithelial cells	Decreased on metastatic cells
Teratocarcinomas	Changes with differentiation
Early embryonic tissues	Cells from all three germ layers have been reported to synthesize fibronectin.

* Specific references can be found in earlier reviews (see 1, 4, 11, and 13 for summary tables with references) and in the text.

TABLE II

*Properties of Fibronectins**

Subunits	220,000 ± 20,000-dalton chains in disulfide-bonded dimer.
pI	5.5–6.3
Carbohydrate	5–9% asparagine-linked complex oligosaccharides.
Sulfhydryl groups	One or two in the C-terminal 30%
Disulfide bonds	~20 per subunit. Intersubunit bond(s) are very near C-terminal. N-terminal 25% is very rich in intrachain disulfides.
Secondary structure	No α helix, probably some β structure.
Tertiary structure	Asymmetric and elongated with globular domains.
High order associations	Form disulfide-bonded complexes and fibrils.

* Specific references can be found in earlier reviews (see 3, 4, and 12 for summary tables with references) and in the text.

not require exogenous fibronectin for adhesion and spreading, but many cells that produce little or none will respond to added fibronectin. Among these cells are some oncogenically transformed cells that produce reduced quantities of fibronectin as a consequence of transformation (37, 38).

Concomitant with the spreading induced by added fibronectin, cells often acquire highly ordered intracellular microfilament bundles (38, 39). Furthermore, extracellular fibrils that contain fibronectin are often observed to correspond in their arrangement with intracellular microfilament bundles (40–47). Fig. 1 shows examples of this phenomenon. These results, and others, suggest that there may be some form of physical connection between the extracellular matrix and the intracellular cytoskeleton (10). The molecular basis for this interaction is not understood as yet. However, the idea that the cytoskeleton and the extracellular matrix are part of a continuous supramolecular assemblage has important implications for considerations of the effects of extracellular matrices on cellular behavior.

Interestingly, oncogenic transformation causes a pleiotropic change in cellular properties including reduced adhesion, rounded morphology, and loss of cytoskeletal organization, as well as loss of fibronectin. Because all of these changes can, in some cases, be reverted by addition of fibronectin (37–39), it is possible that they reflect a common effect of the transforming agent (48). Extensive studies have shown that loss of fibronectin is a common, though not universal, concomitant of transformation (1–13). The reasons for the loss appear to vary, and include reduced synthesis (49, 50), reduced binding (49–53), and increased rates of degradation (49, 54). Correlative studies suggest some association between loss of fibronectin and tumorigenicity in vivo (e.g., 55, 56) but exceptions have been noted (e.g., 57, 58), and preliminary studies suggest that a better correlation may exist between loss of fibronectin and acquisition of metastatic potential (59–61). Given the involvement of fibronectin in adhesion to extracellular matrices, effects on invasion and metastasis might be expected from alterations in this aspect of cellular function.

In vitro experiments have shown that fibronectin will promote cell migration in culture (62, 63) and can stimulate chemotaxis or haptotaxis (64, 65). This raises the possibility that fibronectin might promote or guide cell migration in embryos and, indeed, fibronectin has been found in association with several areas of cell migration including avian gastrula (66), neural crest (67–70), area vasculosa (68), amphibian primordial germ cells (71), and sea urchin primary mesenchyme (72, 73). Perhaps also related to an involvement in cell migration is the stimulation by fibronectin of neurite outgrowth from retinal cell aggregates (74). It should be stressed that the

TABLE III

Interactions of Fibronectins

	Selected references
Gelatin, denatured collagens, collagens I–V	*
Fibrin, fibrinogen	*
Factor XIIIa transglutaminase	*
Heparin	*
Proteoglycans	*
Cells	*
Bacteria	75, 76
Actin	127
DNA	137
Hyaluronic acid	*
Gangliosides	165, 166
Asymmetric acetylcholinesterase	167
Clq component of complement	77, 78, 79
Thrombospondin	97

* See text and reviews for specific references.

TABLE IV

*Functions of Fibronectins**

Cellular adhesion
Cellular morphology and spreading
Cytoskeletal (microfilament) organization
Oncogenic transformation
Cell migration/chemotaxis or haptotaxis
Phagocytosis
Hemostasis/thrombosis
Embryonic differentiation

* Specific references for the various functions are given in the text.

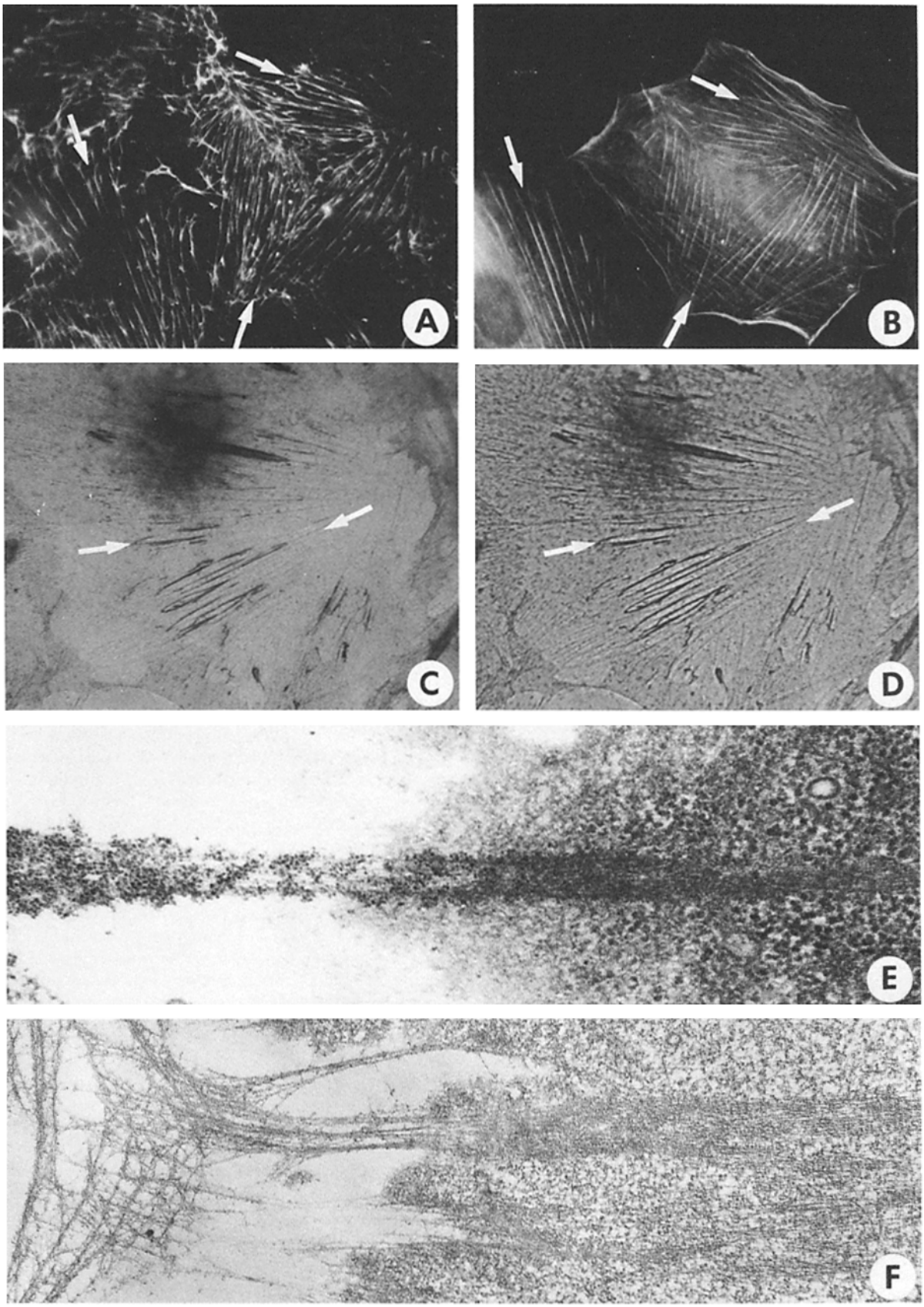

FIGURE 1 Codistribution of fibronectin fibrils and actin microfilament bundles. (*A* and *B*) Double label immunofluorescence of NIL8 hamster cells arrested in low serum (cf. reference 40) *A* shows fibronectin stain, *B* shows actin staining of the same cells. Arrows mark some of the coincidences. (*C* and *D*) Immunoperoxidase staining of fibronectin in *Xenopus* mesentery cell cultures. *C* shows peroxidase staining of fibronectin. *D* shows a phase photograph of the same field. Actin microfilament bundles are colinear with the fibronectin (reprinted from reference 71). (*E*) Immunoferritin staining of fibronectin in tsSV40-hamster embryo fibroblasts at nonpermissive temperature. Figure shows an oblique section through the base of the cell with fibronectin fibril outside cell at left colinear with actin microfilament bundle inside cell at right (reprinted from reference 42). (*F*) Similar section of NIL8 hamster cells not stained with antibody. Figure again shows extracellular fibrils colinear with intracellular microfilament bundles (picture courtesy of Irwin Singer).

presence of fibronectin in areas of cell migration does not prove that it is functionally involved, even less that it is the only relevant matrix component.

A functional role for fibronectin in phagocytosis was originally suggested on the basis of in vivo results that showed that levels of plasma fibronectin correlated with the ability of an organism to clear particulate debris, especially gelatin-coated particles, from the circulation (5). It was suggested that fibronectin was acting as a "non-specific opsonin" for the reticuloendothelial system. This suggestion became even more interesting with the observation that fibronectin binds to certain bacteria (75, 76). The recently reported binding of Clq by fibronectin (77–79) might also be important in phagocytosis of antibody aggregates or fragments of cells lysed by complement. In vitro studies have shown that fibronectin will indeed promote phagocytosis of gelatin-coated beads by certain macrophages although heparin is required as a cofactor (21, 80–82). No detailed studies have yet shown fibronectin to act as an opsonin for bacteria. Thus, the in vivo relevance of the "opsonic" activity of fibronectin remains unclear and is under active investigation. One interpretation of fibronectin-stimulated endocytosis is that it is simply a specialized form of cell spreading on a substratum with a small rather than an infinite radius of curvature.

Another possible role of plasma fibronectin is in hemostasis and thrombosis. During coagulation, fibronectin is crosslinked to fibrin by factor XIIIa transglutaminase (83, 84). Furthermore, platelets contain intracellular fibronectin (85, 86), possibly in their α-granules, and release it on activation (86–88). Activated platelets will also bind exogenous plasma fibronectin (50, 88, 89). Consequently, at the site of interaction of platelets with endothelial cell basement membrane, there are three possible sources of fibronectin; plasma, basement membrane, and the platelets themselves. Because other adhesive proteins such as von Willebrands factor and thrombospondin are also present in the platelets, the basement membrane and the plasma (88, 90–94), the adhesion of platelets is likely to be complex. The exact role of fibronectin in this process remains unclear but, under certain artificial in vitro conditions, fibronectin can promote the adhesion and/or spreading of platelets (50, 95, 97). Crosslinking experiments suggest that an interaction between fibronectin and thrombospondin occurs when platelets spread on solid substrata (97). Thrombospondin has also been implicated in platelet aggregation (98, 99).

Finally, fibronectin has been implicated in the regulation of several differentiation pathways. Fibronectin has been reported both to stimulate myogenesis and to inhibit myoblast fusion (100, 101) and to inhibit chondrogenesis (102–104). The precursor cells of both the myogenic and chondrogenic lineage are fibronectin-positive, and there appears to be a loss of fibronectin during progression along these two differentiation pathways (103–108). Fibronectin has also been reported to inhibit melanogenesis and to promote adrenergic differentiation in explanted neural crest cells (109, 110). Extracellular matrices have long been thought to play important roles in development. Fibronectin has now joined collagens and proteoglycans as a candidate for a role in these processes, as have even more recently discovered glycoproteins such as laminin and chondronectin. Clearly the role of the various matrix constituents, singly and in combination, in various differentiative events will be an active area of research in the next few years.

Fibronectins therefore appear to be involved in an almost embarrassingly large array of cellular functions. How can an individual molecule perform so many functions? The following sections review our current understanding of the structure-function relationships of fibronectins.

STRUCTURE AND FUNCTIONAL DOMAINS OF FIBRONECTINS

As mentioned above, spectrophotometric and ultracentrifugation experiments indicate that cellular and plasma fibronectins are elongated molecules composed of structured domains separated by flexible, extendable regions of polypeptide chain (23, 24, 111). By electron microscopy, fibronectins are usually visualized as slender, elongated molecules with regions of apparently increased flexibility (25, 26), although they can appear more globular under certain conditions (27). Variability also exists in the hydrodynamic radius, which is known to increase or decrease, depending upon ionic strength (23). A unifying interpretation of these studies is that fibronectins are highly flexible molecules that can expand or contract depending upon the local environment.

Flexible polypeptide regions of proteins such as fibronectin tend to be particularly susceptible to attack by a variety of proteases. Proteases will cleave such regions to generate separate, structured domains of the molecule containing specific binding sites for ligands (e.g., collagen and heparin, see Table III).

There are two approaches to purifying such structural domains from fibronectin or any molecule with binding sites. In the first, specific ligands are coupled covalently to agarose beads and are poured into affinity columns. The fibronectin is allowed to bind to the column and then incubated with various proteases. Under appropriate conditions, the enzymes cleave the molecule into protease-resistant domains. After extensive washing of the column, the only regions that remain bound to the column are domains specific for binding to the specific ligand. An alternative approach is to pretreat the fibronectin with proteases, to inhibit the proteases with protease inhibitors, and to pour the entire digest over affinity columns. Specific protease-resistant domains then bind to the column, and other fragments are discarded. These two approaches have been surprisingly successul in identifying and purifying a series of protease-resistant, functional domains of fibronectin and locating these domains and other structural features on the molecule (112–132). Fig. 2 shows the current model based on these results.

Collagen-binding Domain

The first domain of fibronectin to be isolated was the collagen-binding domain. This region of the molecule is ~30–40,000 daltons in size, and can be produced after digestion of the intact molecule with chymotrypsin, subtilisin, thermolysin, or even the broad spectrum protease pronase (112, 114, 116, 117, 119, 120, 124, 125). This region binds to collagen or to gelatin affinity columns, yet cannot mediate cell interactions. Larger fragments are produced by different proteolytic conditions and allow mapping of the collagen-binding domain near to but not at the amino-terminius (112, 113, 115, 116, 118, 119, 120, 122–125, 128–131).

Cell-binding Domain

The cell-binding activity appears to require a separate region, the "cell-binding" region of fibronectin, that can be purified

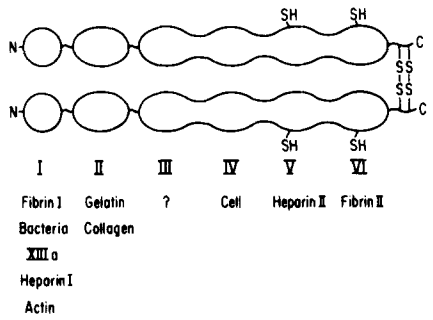

FIGURE 2 A current working model for the modular structure of fibronectins. The molecules are composed of elongated subunits linked by disulfide bonds near the carboxyl terminus. Some regions are extremely sensitive to proteolysis and are depicted as extended chains (thin lines). Other regions are compact and globular and contain specific binding sites for other molecules. Two protease-resistant domains (*I* and *II*) are well-defined and readily purified. The remainder of the molecule is less well-analyzed, but appears to contain regions that are relatively sensitive to proteolysis (constrictions) and other less sensitive regions that can be isolated by virtue of their binding affinities. The locations of the different binding sites on the molecule are indicated. See text for details.

from among the fragments of the molecule that do not bind to collagen affinity columns (112, 116, 120, 125, 128, 129, 132). The cell-binding region of fibronectin can mediate the attachment and spreading of cells on simple plastic substrates, even though it is inactive in mediating the attachment of cells to collagen. A fragment of 15,000 daltons still retains a significant amount of the cell binding activity of the intact molecule, but does not bind to either collagen or heparin (132). This fragment has been isolated using a monoclonal antibody that blocks cell binding activity (132). Analyses of partial digests show that this 15-kdalton fragment and the cell-binding site lie in the central part of the molecule (116, 120, 125, 128, 129, 132).

Fibrin and Transglutaminase Interaction Sites

The binding of fibronectin to fibrin from blood may be important in the initial stages of wound healing (133). Fibroblasts involved in the healing process adhere particularly well to fibronectin cross-linked to fibrin by factor XIIIa transglutaminase (35). Fibronectin binds to fibrin or to fibrinogen both via its amino-terminal domain and through site(s) near the carboxyl terminus of the molecule (128, 134, 135). However, the transglutaminase-mediated cross-linking appears to occur only at the amino-terminal domain to a glutamine residue of fibronectin (119, 120, 128, 130, 136). Collagen can also be cross-linked to fibronectin by transglutaminase (84). Although the primary noncovalent binding of collagen occurs at the adjacent collagen-binding domain, the covalent cross-linking occurs only to the adjacent amino-terminal domain (119).

Several other interesting interactions are also mediated by the amino-terminal domain of fibronectin. This region also binds to the bacterium *Staphylococcus aureus* and to actin (76, 127, 131). Although the physiological consequences of these latter interactions are not yet known, binding to fibronectin might promote phagocytosis of these materials by the reticuloendothelial system as discussed earlier. DNA is also bound at multiple sites on fibronectin, but this interaction appears to be weak and is inhibited by physiological concentrations of calcium (137, 138).

Glycosaminoglycan-binding Domains

As described previously, interactions of fibronectin with heparin and heparan sulfate may play important roles in the uptake of material by macrophages or in the structural organization of the extracellular matrix (21, 30–32, 80–82, 138–143). When examined in vitro, the interactions of fibronectin and heparin appear to be complex, with at least two components of moderately high binding affinity (121).

The results of proteolytic dissection of the fibronectin molecule to identify the regions that bind to heparin affinity columns are also complex. There are two or three distinct regions of the molecule that bind to heparin (125, 126, 128–131). One binding site is located in the amino-terminal domain of the molecule (125, 128, 130, 131). Interaction of this domain with heparin can be modulated by physiological concentrations of calcium (138) and is inhibited by 0.25 M salt (130).

A second binding site is located close to the carboxyl terminus of the molecule (125, 128–131), and its binding is less sensitive to salt (130) and is insensitive to divalent cations (138). The existence of these multiple heparin-binding domains on fibronectin, and their differing sensitivities to divalent cations and salt concentrations, suggest that the interactions of fibronectin with even one ligand can be surprisingly complex and may be modulated by the local microenvironment.

Fibronectin can also bind to the glycosaminoglycan hyaluronic acid, which could affect the interactions of hyaluronic acid with cells or other extracellular matrix molecules (121, 140, 141). The binding by cellular fibronectin is kinetically complex and of moderately high affinity (121). Recent data suggest that fibronectin must exist as an aggregate in order to mediate efficient binding, because nonaggregated cellular or plasma fibronectins coupled to affinity columns bind poorly to hyaluronic acid (144). Because of this latter property, the binding region for hyaluronic acid has not yet been identified.

Disulfides and Sulfhydryls

Similar analyses of fragments of fibronectin have shown that the interchain disulfides connecting the subunits of the fibronectin dimer are very close to the carboxyl terminus (115, 118, 120, 122, 145–147). Both the amino-terminal and the collagen-binding domains are extremely rich in intrachain disulfides (113, 115, 117, 118). The amino-terminal domain contains almost 10% half cystine and probably contains 10 intrachain disulfides. These may be responsible for the compact protease-resistant structure of this domain. The intrachain disulfides of the collagen-binding domain are essential for binding to collagen (113, 115). Each subunit of fibronectin contains at least one sulfhydryl group (115, 120) located ~170,000 daltons from the amino-terminus (122) and probably a second one further towards the carboxyl terminus (122, 148).

If these sulfhydryl groups are alkylated, the binding of fibronectin into the cell surface matrix is inhibited (115, 122). It is possible that these sulfhydryls are involved in the intermolecular disulfide bonding of fibronectin either to other fibronectin molecules or to other cell surface constituents such as proteoglycans.

Structure-Function Model of Fibronectin

The current model for the structure of fibronectin based on these results postulates a series of protease-resistant structural

domains, each of which contains specific ligand-binding activities (Fig. 2). The existence of these multiple, specific domains on fibronectin can begin to explain how this molecule may act in a variety of molecular interactions. Fibronectin can be viewed as a molecule that interconnects a series of cell and matrix components to form macromolecular complexes at the cell surface and in the extracellular matrix (Fig. 3).

In addition, this model makes predictions about the requirements for certain combinations of domains for specific functions. For example, cell-cell interactions would be expected to require a multivalent molecule to enable fibronectin molecules to bind to more than one cell. Monomeric fibronectin fragments do have greatly diminished activity in such an assay (114). On the other hand, the attachment of cells to collagen would be expected to occur with even simple monomers but would require two different binding domains. As predicted, monomeric fragments of 205,000 daltons containing one cell- and one collagen-binding site are high active (114).

It will be of interest to examine the requirements for the different heparin-binding domains in the nonimmune opsonic activity of fibronectin with macrophages, in terms of which domain is required and whether the interaction can be mediated by a monomeric fragment. More complex activities of fibronectin can also be examined with these fragments. For example, the ability of fibronectin to promote migration of fibroblasts has been attributed to only the cell-binding region of the molecule (149, 150).

Role of Fibronectin's Carbohydrates

Fibronectin contains ~5% carbohydrate, consisting solely of "complex" oligosaccharides linked to asparaginyl residues. The synthesis of these oligosaccharide residues on fibronectin can be inhibited by 95–98% with tunicamycin, a relatively specific inhibitor of glycosylation (151, 153). The resultant nonglycosylated fibronectin is secreted in virtually normal quantities, suggesting that the carbohydrate moieties play no role in the secretion of this glycoprotein (152). However, the rate of turnover of carbohydrate-free fibronectin is accelerated by two- to threefold, and this increased rate results in a threefold decrease in total quantities of fibronectin on the cell surface of tunicamycin-treated chick fibroblasts (152).

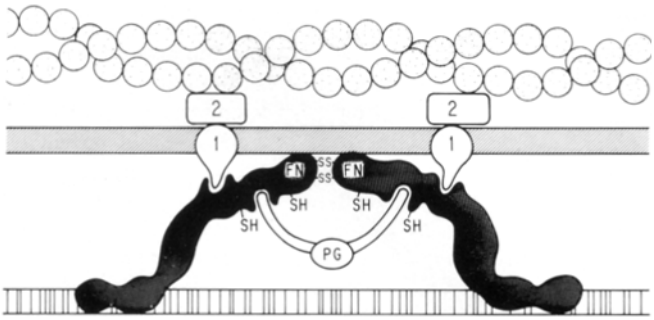

FIGURE 3 Hypothetical scheme showing fibronectin acting as a ligand, connecting the cell to the extracellular matrix. An elonaged dimeric fibronectin molecule is depicted interacting through its N-terminal domains with collagen and through other binding sites with a heparin sulfate proteoglycan (*PG*) and with cells. (cf. Fig. 2). One or more free sulfhydryl groups may also become involved in disulfide-bonding. The nature of the cell surface binding site is unknown—it is depicted here as an integral membrane protein (*1*) but it could be a protein, a glycolipid, or an assemblage of several molecules. The figure also shows one possible form of transmembrane interaction between fibronectin and actin microfilaments, involving a transmembrane "fibronectin receptor" and a molecule binding the microfilaments to the inner face of the membrane (*2*). Candidates for this role include vinculin, α-actinin and/or spectrin. Although the Figure is consistent with known experimental data, the details are speculative.

TABLE V

Plasma Versus Fibroblast Cellular Fibronectins

Action	Relative biological activity*
Cell attachment to collagen	1×
Cell spreading on plastic	1×
Uptake of particles by macrophages	1×
Morphological effects on transformed cells	3–50×‡
Hemagglutination	150–200×§

* Activity of cellular fibronectin compared to plasma fibronectin.

‡ Cellular fibronectin purified from cells by urea extraction shows 50-fold higher activity than plasma fibronectin in this assay (158). Dimeric cellular fibronectin from fibroblast culture medium is at most two- to threefold more active than plasma fibronectin (50). The differences may lie in the degree of polymerization.

§ These values are for cell surface fibronectin (158).

Isolated nonglycosylated fibronectin is unusually susceptible to various proteases (152, 154). The region of fibronectin that becomes the most susceptible to proteolysis is the collagen-binding region, which normally contains most of the carbohydrate residues of glycosylated fibronectin (154). A heparin-binding region of fibronectin that normally lacks carbohydrate has the same protease susceptibility in glycosylated or nonglycosylated fibronectin (154).

Interestingly, there is no evidence that the carbohydrate on fibronectin has any role in its known biological activities. For example, the additional branching of oligosaccharides on fibronectin that accompanies neoplastic transformation has no effect on its morphological activities (53), and even the total absence of carbohydrates after tunicamycin treatment does not alter four other biological activities of fibronectin (153). It therefore appears that the carbohydrates on this molecule function primarily to stabilize specific regions against proteolytic attack and abnormal rates of turnover; this stabilization may also be one of the major functions of carbohydrates on other glycoproteins (155).

Cellular Versus Plasma Fibronectins

Recent studies indicate that the cellular and plasma forms of fibronectin are structurally and functionally very similar, but not identical. The model shown in Fig. 2 apparently applies for both forms. Table V compares the biological activities of purified cellular and plasma fibronectins in a number of in vitro assays. The two forms of fibronectin are indistinguishable in their biological activities in assays involving cell interactions with substrates (156, 157), or in opsonic activity for macrophages (82). In contrast, the effects of these molecules on cell morphology and alignment of transformed cells and on hemagglutination are sometimes substantially different; in these assays, cellular fibronectin can be more active than plasma fibronectin (50, 157).

Biochemical studies have also revealed both similarities and differences between the two molecules. The molecules have very similar amino acid compositions, carbohydrate structures, and secondary and tertiary structures. In addition, the types and the organization of specific structural domains are indistinguishable. Nevertheless, plasma and fibroblast fibronectins have differences in pI (15), solubility (23, 24, 111), numbers of

subunits linked together by disulfide bonds (111, 158–160), and subtle differences in the apparent size of specific domains (113, 131). Any of these differences might be related to the biological differences. An interesting finding in this regard is that the regions of apparent difference between the two forms can be mapped to the interior of the molecules, rather than to an end (131, 161). This finding appears to rule out the previous most popular hypothesis that the two forms are related by simple proteolytic processing of one form to the other. Monoclonal antibodies provide independent evidence that the two forms are distinct, because monoclonal antibodies have been found that bind preferentially to cellular as compared with plasma fibronectin (161, 162). One of these distinguishing monoclonals binds a determinant located near to the carboxy-terminal end but internal to the interchain disulfide bonds and its binding does not appear to require the presence of carbohydrate (161). This suggests the existence of differences in primary sequence.

These studies indicate that although the two forms of fibronectin are very similar, they are structurally and functionally distinct. It will be of considerable interest to determine whether the two forms are the product of two or more distinct genes, or whether they are encoded by a single gene that produces an RNA that is differentially processed to produce mRNA's encoding either cellular or plasma fibronectins.

CONCLUSIONS AND FUTURE PROSPECTS

Analysis of the structure of fibronectins reveals a modular structure that appears well suited for functioning as an adhesive ligand-like molecule. Fig. 3 shows one current view of fibronectin in the role of a cell-matrix ligand and also depicts the possible relationship with intracellular microfilaments. Obviously, several features of this model remain uncertain. However, the general picture of a molecule with binding sites specific for some cell surface molecule(s) connected to other binding sites specific for various extracellular moieties is well-founded on experimental data. Furthermore, some form of relationship with intracellular microfilaments is in accord with current data (10, 38–48), although the molecular basis for the transmembrane relationship remains unknown.

This picture of an involvement of fibronectin in a connection between the cytoskeleton and the extracellular matrix, and in cellular interactions with this matrix, suggests plausible and testable hypotheses to explain the roles played by fibronectins in the various cellular properties discussed earlier (Table IV). The challenge now is to test these hypotheses and resolve the unanswered questions.

Although much is known about the structure (Fig. 2), not all the interactions of fibronectin (Table III) can yet be assigned to specific locations within the molecule, and the details of many of the interactions remain to be analyzed. Further work along the lines discussed earlier should provide much of this information. Detailed structural studies (primary sequence, biophysical measurements) on the individual domains should be available in the near future. Acquisition of primary sequence data will be greatly aided by nucleic acid cloning studies now in progress. Detailed protein structural and nucleic acid analyses will also provide further insight into the nature of the differences between plasma and cellular fibronectins and the possibility of multiple forms of fibronectin.

The modular structure of fibronectin lends itself well to the development of antibodies specific for different functional regions. Several monoclonal antibodies are already available (132, 161, 162), as well as some domain-specific polyclonal antisera (112, 163, 164). In the future, these reagents should be useful for investigating which local domains and binding sites function in which of the more complex biological functions (Table IV). The combination of a detailed analysis of the structure of fibronectins with the development of antibodies able to interfere with specific functions should provide a clear picture of the structure-function relationships of these complex proteins.

Two important properties of fibronectin that remain enigmatic are its ability to form fibrils and its interaction with cells. Analysis by chemical cross-linking, antibodies, and other techniques should allow identification of the cell surface binding site(s) and of the arrangement of fibronectin and other molecules in the fibrils.

The suggested roles of fibronectins in complex biological phenomena such as cell migration, cellular differentiation, hemostasis and thrombosis, reticuloendothelial clearance, and cancer all require much more extensive investigation. Studies of these phenomena in vivo will be contingent upon progress on the biochemical, immunological, and cell biological analyses of fibronectins. Furthermore, because fibronectins undoubtedly interact in vivo with other molecules (collagens, proteoglycans), these other constituents will have to be studied in parallel. It also seems clear that several other proteins of a type similar to fibronectins also function as cell-matrix ligands (eg, laminin, chondronectin, von Willebrands factor, thrombospondin) and this list is likely to grow rapidly. The complex interaction among these various molecules are likely to provide a fertile field for research in the next few years, as they have in the last several.

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